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THE BINDING OF SOLUBLE ANTIGEN-ANTIBODY COMPLEXES TO ERYTHROCYTES

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As investigations by Sorkin [1] and Weigle [2] have shown, cellular blood components are essentially concerned in the elimination of antigen-antibody complexes from the circulation. For leucocytes and thrombocytes it has been shown in this connection that they either phagocytize soluble antigen-antibody products developed in the blood plasma or adsorb them to their surface and suffer injuries in the process which bring about cell destruction [3,4].

The present article is intended to make a contribution to the question of what part the erythrocytes are capable of playing in the elimination of soluble antigen-antibody complexes. We started with the suggestion made by Duesberg [5] that a cleansing function in the circulating blood is to be attributed to the erythrocytes on the ground of the multivalent combining power of their large total surface and their consequent adsorption capacity. As earlier articles have been able to show, the adsorption to the surface of the erythrocytes is a quite specific process, as has been demonstrated for heterogenous macromolecular substances such e.g. as bacterial lipopolysaccharides [6] or myxoviruses [7] as well as for homologous plasma proteins [8,9,10,11].

By a technique which has already been applied to the study of the normal plasma protein film on the surface of human erythrocytes [9], we have tested here to what extent an adsorption of soluble antigen-antibody complexes to the surface of rabbit erythrocytes takes place. These soluble immune complexes were always produced in antigen excess. Human albumin and rabbit antibodies served as a model system. In the experimental series sometimes the antigen and sometimes the antibody was marked with iodine^{131}. For comparison

we used the adsorption quota of the individual components of the immune complex (iodine^{131}-tagged human albumin and iodine^{131}-tagged rabbit gamma globulin)

Material

1. Iodine^{131}-tagged rabbit antibody against human albumin was prepared by the following method: Rabbits were sensitized against human albumin (Behring-Werke, Marburg) with Freund's adjuvant. Blood and serum containing antibody were obtained by heart puncture. The rabbit gamma globulin was precipitated in 1.75 m ammonium sulfate solution, dissolved in Sörensen's phosphate buffer, pH 8.0, and tagged by a slightly modified Pressman and Sternberger [12] method with iodine^{131} (Radiochemical Centre, Amersham). From the solution thus treated, at the equivalence point of the nephelometric Heidelberger curve, which is characterized by a turbidity maximum [13,14], a specific albumin-antialbumin-antibody precipitate was precipitated out with the calculated amount of albumin, repeatedly washed with physiological NaCl solution to eliminate in large part any free iodine components, dissolved in 0.01 n HCl, and separated into albumin and antibody in a column with "Sephadex G 100" (height of column 65 cm) [15,16]. In the first fractions the antibody protein, which is present in pure form in the solution, is concentrated, after the albumin always present in small quantities after neutralization with antibodies and precipitate formation has precipitated out. From the last fractions it was possible after neutralization to obtain a soluble antigen-antibody complex, the gamma globulin-antibody content of which was determined from the measured impulse counts of activity measurement. Excess albumin is always obtained with such an antigen-antibody complex for reasons inherent in the method. For purely formal reasons two molecules of albumin were used per molecule of gamma globulin in view of the confidently assumable bivalence of the antibody molecule [7], so that the soluble antigen-antibody complex is made up of 1 molecule of antibody and 2 molecules of antigen.

2. "Very pure" unmarked human albumin was obtained from the Behring-Werke, Marburg.

3. Iodine^{131}-tagged human albumin came from the Radiochemical Centre, Amersham; it was dialyzed to remove free iodine 24 hours at 4°C against physiological NaCl solution before use.

4. The preparation of soluble antigen-antibody complex was done by adding the quantity of antibody-bearing rabbit serum computed by a Heidelberger curve to the iodine^{131}-tagged human albumin. Here again we must reckon with free albumin which is not bound in the antigen-antibody complex.

Table I. Specific Surface Activity of an Immune Complex in Comparison with Its Individual Constituents.

1	2	3	4	5	6	7	8
No.	Protein	Equilibrium Amount % P_G	Absolute Amount of Protein mg G_B	Number of Erythrocytes $x10^{10}$ Z_B	Protein on the Erythrocyte Surface gamma G_{BE}	Molecule on the Erythrocyte M_{BE}	Factor of Concentration P
1	Iodine^{131}	0.0749	30	2.0	22.47	9667	
2	Human	0.0800	30	2.0	24.00	10520	
3	Albumin	0.0884	30	1.5	26.52	15153	
Av.						11780	
4	Iodine^{131}	0.2230	30	1.5	66.90	38227	3.25
5	Human Albumin	0.3426	30	1.5	102.78	58728	4.99
6	Rabbit	0.2730	30	2.0	81.90	35234	2.99
7	Antibody	0.5733	30	1.5	173.26	99001	8.40
8	Complex	0.6314	30	1.5	189.42	108234	9.19
9	Iodine^{131} Rabbit γ Globulin Antibody	1.746	16.3	0.9	284.35	121395	
10	Human	4.406	147.8	0.9	1338.24	1472605	12.13
11	Albumin Iodine^{131} Rabbit ibody lex	2.808	147.8	0.9	2400.99	938505	7.43

Method

Three ml each of the antigen-antibody complex solutions thus prepared with known immune-complex content (Table I, Column 4), with the antibody in one case and the antigen in the other tagged with iodine^{131}, and also of pure iodine^{131} tagged antibody solutions without albumin and albumin solutions without antibody, were incubated with 2 ml of an erythrocyte suspension from the rabbit for two hours at room temperature and then washed with a Sörensen phosphate buffer solution, pH 7.2, to which physiological NaCl solution had been added in a 1:1 ratio, by the following method: The 5 ml of whole blood solution was mixed with 5 ml of buffer solution, centrifuged for three minutes at 1200 g, and 5 ml of upper stratum piped off in each case. This washing process was repeated ten times. The radioactivities in the various wash waters and in the erythrocyte sediments were measured. For this purpose a scintillation counter with a drilled-out crystal was used, made by Frieseke & Höpfner, Erlangen.

Results

By the equilibrium amount P_G (Table I, Column 3) in % of the original amount of the protein in question is meant the activity and so, the relationship between activity and protein content being known, the absolute quantity of proteins obtained when equal impulse numbers were found in the liquid and the erythrocyte sediment (which also always contained a certain proportion of the liquid). From this in accordance with the scheme used in an earlier article [9] the number of molecules or of immune complexes per individual erythrocyte (M_{EE}, Table I, Column 7) could be computed from the number of erythrocytes in the starting material (Z_E, Table I, Column 5) when the total amount of protein put in (G_B, Table I, Column 4) was known. The concentration factor F is found as the quotient of the number of individual molecules of human albumin or rabbit gamma globulin and the corresponding number of immune complexes which are adsorbed to the surface of the erythrocytes under equal conditions. As is evident from Table I, widely differing M_{EE} numbers were found as between samples 1-3 and 9 and between 4-8 and 10-11. This must be considered as due to the methodologically conditioned fact that in the studies of 1-8 iodine^{131}-tagged human albumin from the Radiochemical Centre, Amersham, was used, and in samples 9-11 human albumin from the Behring-Werke, Marburg, was used. In the iodine^{131}-tagged human albumin from Amersham considerable denaturation phenomena were observed which could not be found in the same degree in the corresponding albumin from the Behring-Werke. As control studies showed, the adsorption of proteins is largely conditioned by their provenience. ([Note] Unpublished experiments.) But since only samples 1-3 were compared with 4-8

and 9 with 10-11, the differing behavior plays no part in the present considerations.

Discussion

The results show that both the heterologous albumin as antigen and the homologous gamma globulin as antibody in the form of an immune complex are bound more strongly to the surface of the erythrocytes than the corresponding proteins dissolved in the free state. The soluble antigen-antibody complex formed thus possesses with respect to the erythrocyte surface a greater tendency to adsorption, which is defined as specific surface activity [18], than the individual proteins of which the immune complex is composed. As shown by Table I, it is greater by a factor of 3 to 12 than in the components from which the soluble complex is made up. From this finding the conclusion may be drawn with great probability that the erythrocytes within the circulating blood perform a cleansing function with regard to soluble antigen-antibody complexes formed. Their capacity for a specific adsorptive bond with such substances may be regarded as evidence for their corresponding function. Whether the corpuscles themselves suffer damage in the process and fall prey to an accelerated sequestration, as is obviously the case especially in allergic-hemolytic conditions, must remain an open question.

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Summary

The surface of erythrocytes adsorbs soluble antigen-antibody complexes to a greater extent than it does either of the two components of the complex separately. Human albumin and rabbit antihuman-albumin antibody were investigated as a model of a soluble antigen-antibody complex. The specific concentration of the immune complex at the red cell surface suggests that these cells are actively involved in the removal of antigen-antibody complexes from the circulation.

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